

REMARKS

The specification has been amended to comply with the Sequence Listing requirements of 37 CFR §§1.821 *et seq.* Specifically, page 11 of the specification was amended such that SEQ ID NO:3 and SEQ ID NO:4, set forth in Fig. 2, and SEQ ID NO:27 and SEQ ID NO:28, set forth in Fig. 10c, are now referenced.

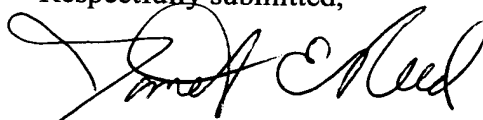
Additionally, this amendment directs replacement of the originally filed paper copy of the Sequence Listing with the paper copy of the amended Sequence Listing submitted herewith. The Sequence Listing was amended to include SEQ ID NOS: 19-28, which were set forth in the specification and drawings as filed, but which had not been included in the originally filed Sequence Listing. Hence, no new matter has been added by this amendment.

A computer-readable form of the amended Sequence Listing is also submitted herewith. Applicants' undersigned attorney verifies that the sequences set forth in the computer-readable form of the Sequence Listing are the same as those set forth in the paper copy of the Sequence Listing.

Applicants submit that the present application is in full compliance with all requirements of 37 CFR §§1.821 *et seq.* and is now in condition for examination on its merits.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

Respectfully submitted,



Janet E. Reed

Registration No. 36,252

Date: *March 11, 2002*

WOODCOCK WASHBURN LLP  
One Liberty Place - 46<sup>th</sup> Floor  
Philadelphia, PA 19103  
(215) 568-3100

DOCKET NO.: UNMC-0027/63116US.1



COPY OF PAPERS  
ORIGINALLY FILED PATENT

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**Sequence Listing as amended:**

SEQUENCE LISTING

<110> Tracy, Steven M.  
Chapman, Nora M.  
Kolbeck, Peter  
Malone, James

<120> Cocksackievirus Vectors And Their Use In Prevention And Treatment Of Disease

<130> UNMC0027 [UNMC 63116]

<140> 09/817,748

<141> 2001-03-27

<150> 09/403,672

<151> 2000-03-27

<150> PCT/US98/04291

<151> 1998-03-05

<150> 08/812,121

<151> 1997-03-05

<160> 28 [18]

<170> PatentIn version 3.1 [FastSEQ for Windows Version 3.0]

<210> 1

<211> 85

<212> DNA

<213> Cocksackievirus

<400> 1

atcactacaa tgacaaatac gggcgcatth ggacaacaat caaggggcag cgtatgtggg	60
gaactacagg gtaatgggtc tcaac	85

<210> 2

<211> 80

<212> DNA

<213> Cocksackievirus

<400> 2

tactcgatca ctacaatgac aaatacgggc gcatttggac aacaatcagg ggcagcgtat	60
gtggggaact acagggtagt	80

<210> 3

<211> 34

<212> PRT

<213> Cocksackievirus

&lt;400&gt; 3

Ser Gly Val Thr Thr Thr Arg Gln Ser Ile Thr Thr Met Thr Asn Thr  
1 5 10 15

Gly Ala Phe Gly Gln Gln Ser Gly Ala Val Thr Leu Glu Met Pro Gly  
20 25 30

Ser Ala

&lt;210&gt; 4

&lt;211&gt; 25

&lt;212&gt; PRT

&lt;213&gt; Cocksackievirus

&lt;400&gt; 4

Met Lys Ser Asn Ser Ile Thr Thr Met Thr Asn Thr Gly Ala Phe Gly  
1 5 10 15

Gln Gln Ser Gly Ala Val Tyr Val Gly  
20 25

&lt;210&gt; 5

&lt;211&gt; 27

&lt;212&gt; DNA

&lt;213&gt; Cocksackievirus

&lt;400&gt; 5

atgggaaatt cgagctcgat gcctggc

27

&lt;210&gt; 6

&lt;211&gt; 28

&lt;212&gt; DNA

<213> Cxsackievirus

<400> 6  
atgaaaagcg catgcgggtt ttcaaggt

28

<210> 7

<211> 74

<212> DNA

<213> Cxsackievirus

<400> 7  
actactaggc aaagcatcac tacaatgaca aatacgggcg catttggaca acaatcaggg  
cagtctcgga tcca

60

74

<210> 8

<211> 62

<212> DNA

<213> Cxsackievirus

<400> 8  
gaattctgca gatcaattac caccatgacc aacacggggc gcatttggac aatcaggggc  
ag

60

62

<210> 9

<211> 5

<212> PRT

<213> Cxsackievirus

<400> 9

Asn Thr Gly Ala Phe

1

5

<210> 10

<211> 11

<212> PRT

<213> Cocksackievirus

<400> 10

Tyr Arg Val Met Gly Leu Asn Tyr Ser Ile Thr  
1 5 10

<210> 11

<211> 58

<212> PRT

<213> Cocksackievirus

<400> 11

Ser Gly Val Thr Thr Thr Arg Gln Ser Ile Thr Thr Met Thr Asn Thr  
1 5 10 15

Gly Ala Phe Gly Gln Gln Ser Gly Ala Val Thr Leu Glu Asp Pro Arg  
20 25 30

Val Pro Ser Ser Asn Ser Ile Thr Thr Met Thr Asn Thr Gly Ala Phe  
35 40 45

Gly Gln Gln Ser Gly Ala Val Tyr Val Gly  
50 55

<210> 12

<211> 59

<212> PRT

&lt;213&gt; Cocksackievirus

&lt;400&gt; 12

Ser Gly Val Thr Thr Thr Arg Gln Ser Ile Thr Thr Met Thr Asn Thr  
1 5 10 15

Gly Ala Phe Gly Gln Gln Ser Gly Ala Val Thr Leu Glu Met Pro Gly  
20 25 30

Ser Ala Met Lys Ser Asn Ser Ile Thr Thr Met Thr Asn Thr Gly Ala  
35 40 45

Phe Gly Gln Gln Ser Gly Ala Val Tyr Val Gly  
50 55

&lt;210&gt; 13

&lt;211&gt; 69

&lt;212&gt; DNA

&lt;213&gt; Cocksackievirus

&lt;400&gt; 13

atgggaaatt cgagctccgt acccggggat cctctagagt cgacctgcag gcatgcgggt 60

tttcaagga 69

&lt;210&gt; 14

&lt;211&gt; 55

&lt;212&gt; DNA

&lt;213&gt; Cocksackievirus

&lt;400&gt; 14

atgggaaatt cgagctcgat gcctggcatg aaaagcgcat gcgggttttc aaggt 55

&lt;210&gt; 15



<211> 23

<212> PRT

<213> Cocksackievirus

<400> 15

Met Gly Asn Ser Ser Ser Val Pro Gly Asp Pro Leu Glu Ser Thr Cys  
1 5 10 15

Arg His Ala Gly Phe Gln Gly  
20

<210> 16

<211> 18

<212> PRT

<213> Cocksackievirus

<400> 16

Met Gly Asn Ser Ser Ser Met Pro Gly Met Lys Ser His Ala Gly Phe  
1 5 10 15

Gln Gly

<210> 17

<211> 138

<212> DNA

<213> Cocksackievirus

<400> 17

actactaggc aaagcatcac tacaatgaca aatacgggcg catttgaca acaatcaggg 60  
cgagtctcgg atccagaatt ctgcagatca attaccacca tgaccaaacac cggggcgcat 120

ttggacaatc aggggcag

138

&lt;210&gt; 18

&lt;211&gt; 46

&lt;212&gt; PRT

&lt;213&gt; Cocksackievirus

&lt;400&gt; 18

Thr Thr Arg Gln Ser Ile Thr Thr Met Thr Asn Thr Gly Ala Phe Gly  
 1 5 10 15

Gln Gln Ser Gly Ala Val Ser Asp Pro Glu Phe Cys Arg Cys Ile Thr  
 20 25 30

Thr Met Thr Asn Thr Gly Ala Phe Gly Gln Ser Gly Ala Val  
 35 40 45

&lt;210&gt; 19

&lt;211&gt; 124

&lt;212&gt; DNA

&lt;213&gt; Cocksackievirus

&lt;400&gt; 19

catcactaca atgacaaata cgggcgcatt tggacaacaa tcaggggcag tctcggatcc 60

taggatatcc tgcaggatta caactatgac taacaccggg gctttcggtc agcagagtgg 120

ggca 124

&lt;210&gt; 20

&lt;211&gt; 41

&lt;212&gt; PRT

&lt;213&gt; Cocksackievirus

&lt;400&gt; 20

Ile	Thr	Thr	Met	Thr	Asn	Thr	Gly	Ala	Phe	Gly	Gln	Gln	Ser	Gly	Ala
1				5				10						15	

Val	Ser	Asp	Pro	Arg	Ile	Ser	Cys	Arg	Ile	Thr	Thr	Met	Thr	Asn	Thr
		20						25					30		

Gly	Ala	Phe	Gly	Gln	Gln	Ser	Gly	Ala
	35						40	

&lt;210&gt; 21

&lt;211&gt; 268

&lt;212&gt; DNA

&lt;213&gt; Cocksackievirus

&lt;400&gt; 21

catcactaca atgacaaata cgggcgcatt tggacaacaa tcaggggcag tctcggatcc	60
ggatgaaaaa ggggtgcctc ttccaaaggt tgacttgcaa ttcttctcaa atactacctc	120
tttgaacgac cggcaaggca atgctactaa accaaaagtg gttttgtaca gtgaagatgt	180
aaatatggaa accccagaca cacatctgtc tgactgcagg attacaacta tgactaacac	240
cggggcctttc ggtcagcaga gtggggca	268

&lt;210&gt; 22

&lt;211&gt; 89

&lt;212&gt; PRT

&lt;213&gt; Cocksackievirus

&lt;400&gt; 22

Ile	Thr	Thr	Met	Thr	Asn	Thr	Gly	Ala	Phe	Gly	Gln	Gln	Ser	Gly	Ala
1				5				10						15	

Val Ser Asp Pro Asp Glu Lys Gly Val Pro Leu Pro Lys Val Asp Leu  
20 25 30

Gln Phe Phe Ser Asn Thr Thr Ser Leu Asn Asp Arg Gln Gly Asn Ala  
35 40 45

Thr Lys Pro Lys Val Val Leu Tyr Ser Glu Asp Val Asn Met Glu Thr  
50 55 60

Pro Asp Thr His Leu Ser Asp Cys Arg Ile Thr Thr Met Thr Asn Thr  
65 70 75 80

Gly Ala Phe Gly Gln Gln Ser Gly Ala  
85

<210> 23

<211> 31

<212> DNA

<213> Cocksackievirus

<400> 23

tccgqatgaa aaaggggtgc ctcttccaaa g 31

<210> 24

<211> 30

<212> DNA

<213> Cocksackievirus

<400> 24

gcctctgcag tcagacagat gtgtgtctgq 30

<210> 25

<211> 20

<212> DNA

<213> Coxsackievirus

<400> 25

ctagactctg ccaatacga 20

<210> 26

<211> 21

<212> DNA

<213> Coxsackievirus

<400> 26

gtgctcacta agaggtctct g 21

<210> 27

<211> 49

<212> DNA

<213> Coxsackievirus

<400> 27

catcacaact atgactaaca ccggggcttt cggtcagcag agtggggca 49

<210> 28

<211> 16

<212> PRT

<213> Coxsackievirus

<400> 28

<u>Ile</u>	<u>Thr</u>	<u>Thr</u>	<u>Met</u>	<u>Thr</u>	<u>Asn</u>	<u>Thr</u>	<u>Gly</u>	<u>Ala</u>	<u>Phe</u>	<u>Gly</u>	<u>Gln</u>	<u>Gln</u>	<u>Ser</u>	<u>Gly</u>	<u>Ala</u>
1			5				10						15		

**Amended paragraph beginning at page 11, line 25:**

Figure 2 shows the amino acid sequence of the PLS-CVB3 genome (SEQ ID NO:11) and the mIL-10-CVB3 (SEQ ID NO:12) at the site of the protease 2A cleavage (in SEQ ID NO:12, the sequence to the left of A...M is SEQ ID NO:3 and the sequence to the right of A...M is SEQ ID NO:4). In this construct, the cloning procedure has been modified to include a polylinker site (PLS) to facilitate the use of the CVB3 as a generic cloning and expression vehicle. Further modifications include non-direct repeat genetic sequences to code for the protease P2-A cleavage site in the nascent polyprotein. The amino acids donated by the PLS are underlined, while the amino acids which form the 2A cleavage recognition signal are double underlined. The sequence of the mIL-10 insertion is shown in bold.

**Amended paragraph beginning at page 14, line 27:**

Figure 10 shows PCR and sequence analysis of CVB3-PL2-Ad2L1. pCVB3-PL2-Ad2L1 was transfected into HeLa cells, and the resultant progeny virus (CVB3-PL2-Ad2L1, pass 1) was subsequently serially passaged in HeLa cell cultures (passes 2 to 10). Viral RNA was isolated from virus stocks at each passage, and the presence of the inserted Ad2 sequence was analyzed by PCR using primers flanking the insertion site in the CVB3 genome. Fig. 10 a: amplimers were separated by agarose gel electrophoresis. CVB3-PL2-Ad2L1, RT-PCR amplimer using

chimeric viral RNA as the template; pCVB3-PL2-Ad2L1, PCR amplimer using the chimeric plasmid DNA as the template; CVB3/0, RT-PCR amplimer using the parental CVB3/0 RNA as template; neg., RT-PCR using RNA as template from uninfected HeLa cells; Marker, 100-bp DNA ladder. Fig. 10b and 10c: the sequence of the Ad2 insert-containing 446-bp amplimer (CVB3-PL2-Ad2L1) (Fig. 10b; nucleic acid sequence is SEQ ID NO:21; amino acid sequence is SEQ ID NO:22) and the sequence of the 225-bp Ad2 fragment-deleted amplimer (CVB3-PL2-Ad2L1del) (Fig. 10c; nucleotide sequence is SEQ ID NO:27 [comprises bases 1-49 of SEQ ID NO:21]; amino acid sequence is SEQ ID NO:28 [comprises residues 1-16 of SEQ ID NO:22]) were obtained after isolation of the DNA fragments from agarose gels. Sequence analysis was performed with the same primers as for the RT-PCR analysis. Numbering is based on the CVB3/0 genome (Genbank accession no. M88483).